PROJECT NUMBER : 6902
PROJECT TITLE : Biochemical Special Investigations
PROJECT LEADER : B. D. Davies
PERIOD COVERED : February, 1991

I. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

A. Objective: Develop a nicotine monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) for the determination of nicotine concentrations in samples of interest to PM.

B. Results: The Bio-Tek microtiter plate reader and microtiter plate washer have been received, installed and calibrated. Two experiments were conducted to assure that the plate reader is functioning correctly.

Using the ELISA analysis software supplied with the plate reader, two protocols were written to facilitate the complete analysis of ELISA data.

Several experiments were conducted this month to examine the amount of variation contributed to ELISA analyses by our tobacco extraction procedure. HPLC analyses performed by Project 6912, of replicate extracts of 250 mg of tobacco in 50ml of 1% triethanolamine (TEA) and our standard 500 mg of tobacco in 50 ml of 1% TEA were conducted and compared. The analyses indicated the replicate 250 mg extracts had a lower %RSD than the 500 mg extracts. The use of 250 mg tobacco extracts, with an appropriate change in the standard dilution prior to ELISA analysis, has been incorporated into the assay.

Using this extraction modification on tobacco from a new bottle of Grizzel Bright Monitor supplied by ARD, 18 replicate extractions were prepared and 10 of these were examined using both Nicotine ELISA and HPLC analyses (Project 6912). The average % nicotine value (mg/mg basis) and its associated %RSD for the ELISA data was 2.21% and 8.1%, respectively. For the HPLC data they were 2.20% and 4.29%, respectively. Both of these values were lower than the ARD (E-86b method) value of 2.5% determined for this same sample.

A preliminary experiment was conducted to examine the time course of nicotine extraction using our procedure. The results of that experiment will be reported next month.

C. Plans: Complete the time course experiments. Determine the source of the difference between ELISA and ARD % nicotine values.

D. References:

1. Dunn, R. L. Notebook No. 9049, pp. 14-16.

2. Davies, B. D. Notebook No. 8638, p. 193.

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3. Crockett, E. A. Notebook No. 8998, pp. 191-200.

4. Crockett, E. A. Notebook No. 9061, pp. 1-24.

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